

## Genotyping protocol

EM:12631

**C57BL/6J-Mitf<sup>fmi-enu22(398)</sup>/Kctt**

### Primers

Mitf fw            5' TCA GGG ACG TTA AAC AGG ATA GT 3'  
 Mitf rev           5' CAG TCC ATT GCT AAG GGC GA 3'

### PCR profile

95 °C, 5 min

95 °C, 30 s  
 58 °C, 45 s                            35 cycles  
 72 °C, 45 s

72 °C, 10 min  
 4 °C, ∞

### PCR mix

10 x PCR Buffer II (Life technologies)	2.0 µl
MgCl <sub>2</sub> (25 mM)	1.2 µl
dNTP mix (25 mM of each nucleotide)	0.16 µl
Mitf fw (100 µM)	0.1 µl
Mitf rev (100 µM)	0.1 µl
AmpliTaq Gold (5 U/µl)	0.13 µl
DNA template (~ 0.5 µg tail DNA)	1.0 µl
H <sub>2</sub> O	<u>15.31 µl</u>
	20 µl

### Post-PCR analysis

1.5% agarose gel.  
 Expected pattern is wt and mut: **486 bp**

### Sequencing

Using Mitf fw primer...mutation of C to T

Wild type:

aGCACAATGGCCAGTACCTTACAGGGGATTGATAATCTGTTCTGTTGGTCAACTGGTCAGT  
 CTCACAATCTATGCTACAGGAAAAGCTACCTGAAAGCCCCGAATAACACTGCTATCTTCTC  
 TTCCCTCCACGGCCATGCTCAGGTGCAGACCCACCTGGAAAACCCACCAAGTACCACAT  
 ACAG ■ AAGCTCAGAGGCACCAGGTAAAGCAGTACCTTTCTACCACTTTAGCAAATAAACA  
 TGCCAGCCAAGTCCTGAGCTCACCATGTCCAAACCAGCCTGGCGACCATGCCATGCCACC  
 AGTGCCGGGGAGCAGCGCACCCAACAGCCCTATGGCTATGCTCACTCTTAACTCCAAC TG

TGAAAAAGAGGTAATTCACGTCTCCTCTTCTCCTCTGTTTCTTACATTCCATTA ACTGTCTG  
TGGGATACTCCTCTCGCCCTTAGCAATGGACTGTCTTTGTCCACCTGGTCATTGTCACCAA

Mutant:

aGCACAATGGCCAGTACCTTACAGGGGATTGATAATCTGTTCTGTTGGTCAACTGGTCAGT  
CTCACAATCTATGCTACAGGAAAAGCTACCTGAAAGCCCCGAATAAACACTGCTATCTTCTC  
TTCCCTCCACGGCCATGCTCAGGTGCAGACCCACCTGGAAAACCCACCAAGTACCACAT  
ACAG **I** AAGCTCAGAGGCACCAGGTAAAGCAGTACCTTTCTACCACTTTAGCAAATAAACA  
TGCCAGCCAAGTCCTGAGCTCACCATGTCCAAACCAGCCTGGCGACCATGCCATGCCACC  
AGTGCCGGGGAGCAGCGCACCCAACAGCCCTATGGCTATGCTCACTCTTA ACTCCA ACTG  
TGAAAAAGAGGTAATTCACGTCTCCTCTTCTCCTCTGTTTCTTACATTCCATTA ACTGTCTG  
TGGGATACTCCTCTCGCCCTTAGCAATGGACTGTCTTTGTCCACCTGGTCATTGTCACCAA